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1. APPL.ENVIRON.MICROBIOL 1998, 64;2247-2255

2. APPL.ENVIRON.MICROBIOL 1999 65; 3710-3713

3. APPL.ENVIRON.MICROBIOL 1996, 62; 2994-2998

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Genetic Transformation of *Streptococcus mutans*

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Three strains of *Streptococcus mutans* belonging to serotypes a, c, and f were transformed to streptomycin resistance by deoxyribonucleic acids derived from homologous and heterologous streptomycin-resistant strains of *S. mutans* and *Streptococcus sanguis* strain Challis. Homologous transformation of *S. mutans* was less efficient than heterologous transformation by deoxyribonucleic acids from other strains of *S. mutans*.

Streptococcus mutans, one of a number of microorganisms inhabiting the oral cavity, has received considerable attention due to its role in the development of dental caries (12). A number of investigations have been concerned with the development of a genetic transfer system in these organisms. Recently, LeBlanc et al. (7) reported the transfer of the pAMβ_1 plasmid (2), which codes for erythromycin resistance and lincomycin resistance, from a group F *Streptococcus* species to three species of oral streptococci, *S. mutans*, *Streptococcus sanguis*, and *Streptococcus salivarius*, by a process resembling conjugation. Transfection of *S. sanguis* and a noncariogenic strain of *S. mutans* by phage deoxyribonucleic acid (DNA) from a cariogenic strain of *S. mutans* has also been reported (5, 6). The resulting transfectants exhibited an increased ability to synthesize insoluble polysaccharide and adhere to glass surfaces, properties characteristic of the cariogenic donor strain. Several studies have demonstrated the genetic transformation of *S. sanguis* strain Challis by DNA extracted from a variety of streptomycin-resistant species of oral streptococci, including *S. mutans* (3, 13). However, attempts to obtain reciprocal transformation between strain Challis and *S. mutans* or the other oral streptococci were unsuccessful. This study describes the transformation of three strains of *S. mutans* comprising three different serotypes by DNA derived from homologous and heterologous streptomycin-resistant strains.

Bacterial strains used in this study are listed in Table 1. The *S. mutans* strains were from the collection of H. D. Slade (Northwestern University Medical School, Chicago, Ill.). Many properties of these strains have been studied extensively (4). *S. sanguis* strain Challis was originally obtained from R. Pakula (Toronto University, Toronto, Canada). Spontaneous streptomycin-resistant mutants of *S. mutans* were isolated from brain heart infusion agar (BHI; Difco Laboratories) containing 1.0 mg of streptomycin per ml. The strain Challis mutant, which was resistant to 5.0 mg of streptomycin per ml, has been described previously (8). Stock cultures were maintained at −20°C either in the lyophilized state or in BHI broth containing 50% glycerol. Cells which were used routinely were grown in BHI broth, stored at 4°C, and transferred weekly. DNA was isolated as described previously (11), except that cells were grown in BHI broth containing 20 mM DL-threonine (1) and 20 mM glucose. The transformation procedure essentially was the same as that described for development of high levels of competence in strain Challis (8).

Fifteen strains of *S. mutans* were incubated for 2 h at 37°C in PYG medium (1% proteose peptone, 0.5% yeast extract, 0.1% glucose; pH 7.6) supplemented with 5% heat-inactivated (60°C for 30 min) horse serum (8), and portions were removed and exposed to 10 μg of either *S. mutans* GS5 or Ingbritt DNA per ml. For controls, DNA was substituted with 0.15 M NaCl plus 0.015 M sodium citrate (SSC). After an additional 135 min of incubation, appropriate dilutions of cells were spread in duplicate on BHI agar containing 200 μg of streptomycin per ml. Transformants were scored after incubating the plates at 37°C for 48 h in a GasPak anaerobic system (BBL). Transformation was observed only in *S. mutans* strains HS6, GS5, and MT557, representing serotypes a, c, and f, respectively (Table 1). Strain Challis was carried as a positive control. Positive transformation was obtained with both GS5 and Ingbritt DNAs, although no attempt was made to quantitate the data at this time.

Previous results have shown that the time for the appearance of optimal competence may differ for different transformable strains (9). Therefore, experiments were carried out to determine the time for optimal competence in the three *S. mutans* strains. Cells were incubated in PYG

TABLE 1. *Transformation of different strains of S. mutans to streptomycin resistance by DNA extracted from streptomycin-resistant S. mutans strains GS5 and Ingbritt*

DNA recipient[a]	Transformation
S. mutans	
HS6(a)[b]	+
E49(a)	−
FA1(b)	−
BHT(b)	−
GS5(c)	+
Ingbritt(c)	−
10449(c)	−
B13(d)[c]	−
OMZ176(d)	−
MT703(e)	−
MT557(f)	+
OMZ175(f)	−
AHT(g)	−
OMZ65(g)[c]	−
6715(g)[c]	−
S. sanguis	
Challis	+

[a] Each strain was grown for 2 h, DNA was added to 10 μg/ml, and incubation was continued for 135 min before plating on BHI agar containing 200 μg of streptomycin per ml.

[b] The letters in parentheses indicate serotypes.

[c] These strains were naturally resistant to 200 μg of streptomycin and were assayed for transformants on medium containing 400 μg of streptomycin per ml.

medium, and portions were removed at different times up to 6 h and exposed to homologous DNA. After allowing an additional 135 min of incubation for phenotypic expression, transformants were selected as described above. Appropriate dilutions were also spread on BHI agar to determine the total number of colony-forming units per milliliter. The results indicate that the time for appearance of optimal competence did indeed differ among the three *S. mutans* strains (Fig. 1). Strain MT557(f) was optimally competent after 2.5 h, whereas optimal competence in strain GS5(c) occurred after 5 h of incubation. Interestingly, two peaks of optimal competence occurred in strain HS6(a), one after 3 h and a second after 5 h of incubation. Although the difference in transformation frequencies was relatively small, these peaks were nevertheless reproducible. Perhaps an examination of single-colony isolates would reveal whether this is an inherent property or whether strain HS6 is composed of a mixed population with respect to competence development. Competence occurred during the exponential growth phase in all three strains as previously noted for strain Challis (11). The differences in time of optimal competence in *S. mutans* strains may be related to differences in the rate of growth and the size of the cell population. Although optimal competence in strains MT557 and GS5 differed by 2.5 h, the number of colony-forming units per milliliter at these times was about the same (2.6×10^7 to 3.0×10^7). The growth rates of strains GS5 and HS6 were considerably slower than that of strain MT557, even though the initial inocula of the former strains were approximately twice as large. Maximal transformation occurred in all three strains after approximately 20 min of exposure to DNA (data not shown).

Reciprocal transformation reactions among the transformable strains of *S. mutans* and strain Challis were also performed. Competent cells were incubated with homologous or heterologous DNA (10 μg/ml) for 20 min, deoxyribonuclease (100 μg/ml) was added, and incubation was continued for 120 min before plating. Controls contained DNA and deoxyribonuclease added simultaneously. The results show that strain Challis exhibited the highest level of transformation regardless of whether homologous or heterologous DNA was used (Table 2). As was expected, the homologous transformation of strain Challis was approximately 100-fold more efficient than heterologous transformation (10). The *S. mutans* strains were transformed by DNA from all of the five donor strains to some

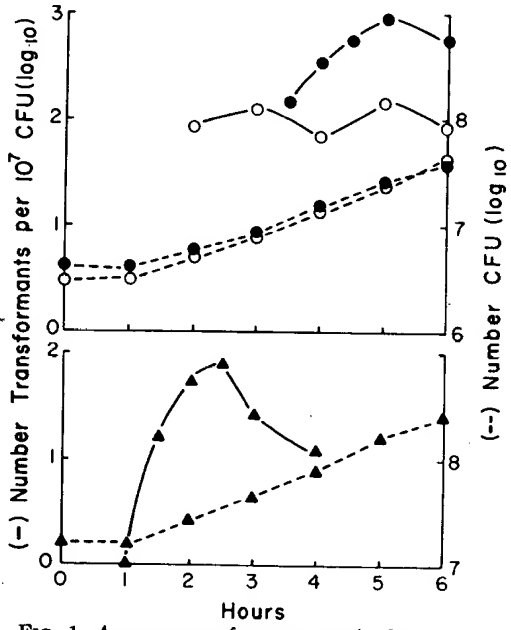

FIG. 1. *Appearance of competence in three strains of S. mutans in relation to time of incubation. Each strain was exposed to DNA from the homologous streptomycin-resistant strain. Symbols: ●, strain GS5; ○, strain HS6; ▲, strain MT557. CFU, Colony-forming units.*

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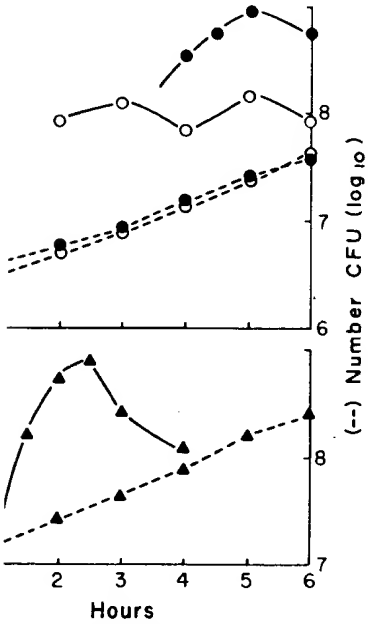

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TABLE 2. *Transformation of three strains of S. mutans and the Challis strain of S. sanguis by homologous and heterologous DNAs*[a]

Source of transforming DNA	No. of transformants per 10^7 CFU[b] with following recipient strain:			
	HS6[c]	GS5	MT557	Challis[d]
S. mutans				
HS6	63	489	354	3,150
GS5	103	414	624	3,250
Ingbritt	142	940	463	4,500
MT557	134	768	53	1,420
S. sanguis				
Challis	63	38	18	265,000

[a] Each strain was grown to optimal competence (Fig. 1) and exposed to 10 μg of DNA per ml for 20 min.

[b] Corrected for controls. CFU, Colony-forming units. The rates of spontaneous mutation to streptomycin resistance of control cells ranged from $0/10^7$ CFU (Challis) to $38/10^7$ CFU (GS5).

[c] Strain HS6 was incubated for 3 h.

[d] Strain Challis was incubated for 2 h.

degree, with DNA from strain Challis being the least efficient. Surprisingly, homologous transformation of *S. mutans* strains was less efficient than heterologous transformation, except when DNA from strain Challis was employed. In the case of strain HS6, homologous transformation was no greater than the heterologous transformation of this strain by DNA from strain Challis. Ingbritt DNA was the most efficient of the four *S. mutans* DNAs in the transformation of strains HS6, GS5, and Challis, whereas strain MT557 was most efficiently transformed by GS5 DNA.

To our knowledge, these results represent the first demonstration of genetic transformation in *S. mutans*. Reasons for the greater efficiency of heterologous over homologous transformation in these strains is presently unknown. Reciprocal transformation (0.0005 to 0.0094%) was obviously low, although reproducible when compared with the transformation of strain Challis (0.14 to 2.7%). It is most probable that conditions for transformation of *S. mutans* were not optimal since the procedure originally was designed for strain Challis. Current studies are aimed at defining the optimal conditions for the transformation of *S. mutans*.

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